# Molecular Genetic Characterization of Neuroendocrine Lung Cancer Cell Lines

SHINN-LIANG LAI[1], HILTRUD BRAUCH[3], TURID KNUTSEN[2], BRUCE E. JOHNSON[1], MARIAN. M. NAU[1], TETSUYA MITSUDOMI[1], CHUN-MING TSAI[1], JACQUELINE WHANG-PENG[2], BERTON ZBAR[3], FREDERICK J. KAYE[1] and ADI F. GAZDAR[1-4]

[1]*NCI-Navy Medical Oncology and* [2]*Medicine Branches, National Cancer Institute, Bethesda, Maryland;*
[3]*Laboratory of Immunology, National Cancer Institute, Frederick 2 Cancer Research Facility, Frederick, Maryland;*
[4]*Simmons Cancer Center and Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.*

**Abstract.** *Small cell lung cancers express neuroendocrine (NE) cell features, while most non-SCLC tumors lack these features. We studied the cytogenetic and genetic alterations in cell lines derived from three unusual subtypes of lung cancer: including carcinoids, non-small cell lung cancers expressing NE properties (NSCLC-NE) and extrapulmonary small cell cancers (ExPuSC) and compared them with those of SCLC and NSCLC lines. Our studies included: cytogenetic studies, restriction fragment length polymorphism (RFLP) analyses with 8 probes spanning commonly deleted loci on chromosomes 3p, 13q and 17p, retinoblastoma gene product (RB) expression, and mutations in the ras and p53 genes. We also summarize previously published data on in vitro chemosensitivity patterns and MDRl gene expression. Our studies demonstrate that all three of the NE cell subtypes have their own distinctive genotypes and phenotypes, each having some similarities and dissimilarities with SCLC and NSCLC.*

A number of clinical and biological features distinguished small cell lung cancers (SCLC) from non-small cell lung cancers (NSCLC)(1,2). Most SCLC tumors are imtially chemosensitive, with response rates of approximately 80%, while most NSCLC tumors exhibit de novo resistance (3). SCLC is a neuroendocrine (NE) tumor, characterized by the presence of several neuroendocrine features including dense core granules and high levels of the key amine handling enzyme: L-dopa decarboxylase (2). Among the NSCLC tumors, only about 15% exhibit the same range of NE markers (NSCLC-NE) (2,4). Bronchial carcinoid is a relatively well differentiated, chemoresistant NE tumor. In addition, tumors morphologically similar to SCLC may arise from extrapulmonary locations, and are termed extrapulmonary small cell cancer (ExPuSC) (5,6). Approximately half of the ExPuSC tumors express NE markers. Previous studies showed that their *in vivo* and *in vitro* chemosensitivity profiles are similar to that of SCLC (5,7,6).

The common human epithelial tumors are characterized by loss of genetic material, either sporadic or specific, at multiple sites. Loss of genetic material at specific sites presumably represents inactivation of known or putative tumor suppressor genes by mutation or deletion (8). Cytogenetic and restriction fragment length polymorphism (RFLP) studies have localized certain sites on the human genome where genetic loss occurs frequently in SCLC, and less frequency in NSCLC (9-16). The sites involved in lung cancer included a large segment of chromosome 3p, 13q14 (Retinoblastoma gene locus), 17p13 (p53 gene locus). However, multiple other sites may also be involved (17-21).

We have demonstrated that cell lines derived from carcinoids, non-SCLC tumors expressing NE markers and ExPuSC carcinomas represent distinct phenotypes (22). In addition, our previous studies suggested that the genetic alterations accompanying ExPuSC may differ from those in SCLC (23). To further understand the relationship of these unusual NE tumors arising in pulmonary and extra-pulmonary sites to the more common lung cancers, we compared their cytogenetic and molecular findings with SCLC and NSCLC.

## Materials and Methods

Cell lines. Six NSCLC-NE, five bronchial carcinoids, and four ExPuSC cell lines were established, maintained and characterized in NCI-Navy Medical Oncology Branch, as described previously (1,24,22).

Cytogenetic analysis. Cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum. Cytogenetic analyses were performed using a modified trypsin-Giemsa banding technique. The cell lines were harvested after incubation at 37 °C with colcemid (0.05 μg/ml) for 3-6 hours. The cells were then treated in a hypotonic solution consisting of 1% sodium citrate and 0.075 M potassium chloride (1:1) for 20 minutes and fixed in a mixture of methanol and glacial acetic acid (3:1). Air-dried chromosome preparations were made. After staining, metaphases were

*Correspondence to:* Dr. Adi F. Gazdar, Simmons Cancer Center, Southwestern Medical Center, Dallas, Texas 75235-8593, USA.

*Key Words:* Neuroendocrine lung cancer cell lines, molecular genetic characterization.

0250-7005/95 $2.00+.40

Table I. *Frequencies of chromosomal abnormalities* in lung cancer cell lines.*

| Tumortype | Chromosome 3p abnormalities (%) | Chromosome 13q abnormalities (%) | Chromosome 17p abnormalities (%) |
|---|---|---|---|
| SCLC (53) | 48 (91%) | 36 (68%) | 34 (53%) |
| NSCLC (74) | 60 (81%) | 24 (32%) | 62 (84%) |
| Carcinoid (4) | 4 (100%) | 3 (75%) | 2 (50%) |
| NSCLC-NE (5) | 0 (0%) | 1 (20%) | 0 (0%) |
| ExPuSC (5) | 2 (40%) | 1 (20%) | 0 (0%) |

Chromosomes 3p, 13q and 17p were scored as abnormal if they had deletions (interstitial, of the whole arm or of the whole chromosome), or if they were involved in translocations (including reciprocal and non-reciprocal translocations, inversions and isochromosomes). Increased numbers of normal appearing chromosomes were not scored as abnormal.

scored for modal chromosome number and aberrations, and G-banded karyotypes were prepared and scored according to the International System of Human Cytogenetic Nomenclature (25).

*RFLP analysis.* DNA extraction, digestion with appropriated restriction enzymes, Southern blotting, hybridization, and evaluation of the results were performed as described previously (10). We analyzed lung cancer cell lines with eight polymorphic DNA probes, homologous to loci on chromosome 3p, 13q, and 17p listed in Table I (26-28) (29-32).

*RB protein expression.* For immunoblotting, cellular protein lysates were prepared from 80% confluent cells in lysis buffer (1% Nonidet p-40/100 mM NaCl 2 mM EDTA/20 mM Tris, pH 8.0) containing phenylmethylsulfonyl (0.01%), aprotinin (1 µg/ml), leupeptin (1 pglml) NaF (5 mM), and sodium orthovanadate (1 mM) at 0°C for 30 minutes. Lysates were cleared by centrifugation at 15.000 x g for 15 minutes and stored at -80 °C. Protein concentration were determined by the Bio-Rad protein assay. Samples (100 µg) were analyzed by SDS/PAGE followed by immunoblotting (33). The mouse monoclonal antibody Mh-Rb-02 (PharMingen, San Diego, CA, USA) were used to detect RB protein. An $^{125}$I-labeled rabbit anti-mouse antibody (Amersham, Arlington, IL, USA) was used for detection.

*Detection of p53 gene mutations.* Point mutations in the p53 gene were detected as described previously (34). Briefly, 100 ng of genomic DNA was amplified in a volume of 10 µl containing 50 mM potassium chloride, 10 mM Tris-HCl (pH 8.3), 1.5 mM magnesium chloride, 0.01% (W/V) gelatin, 1.25 mM each of four dNTPs (Pharmacia), 0.05 µg of a pair of primers, 0.25 units of Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA) and 0.5 µl of [$\alpha$-$^{32}$p]dCTP (300 Ci/mmol$^{-1}$, 10 mCi mmol$^{-1}$, Amersham, Arlington, IL, USA). The amplification reaction using a thermal cycler (Perkin-Elmer Cetus) consisted of 94 °C for 10 min for initial denaturation when using genomic DNA, followed by 35 cycles of 94 °C for 1 min, annealing for 2 min at 55°C, and extension at 72 °C for 2 min.

Following PCR, 1 µl of PCR product was digested for 2 h with appropriate restriction enzymes. Two microliters of the enzyme digestion was transferred to a 96-well plate, mixed with loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue) and incubated in a 90 °C water bath for 5 min. After heating, the samples were immediately cooled on ice and 2 µl of each sample was loaded onto a 6% acrylamide gel containing 89 mM Tris-borate, 2 mM EDTA, pH 8.3 (1 x TBE). The gel was run at 25 W for 5 h in the cold room (4°C) using 1 x TBE as running buffer. After electrophoresis, the gel was dried and exposed to Kodak X-omat AR film with an intensifying screen at -70°C for 15 h.

Mutations detected by SSCP were confirmed by direct sequencing of the PCR product was performed as described previously (34). Briefly, DNA segments containing the mutation which had been localized by SSCP analysis were amplified by PCR using an appropriate pair of primers. Using 1 µl of the product, second-round PCR was carried out with a pair of heminested primers. The product was purified by electrophoresis using low melting point agarose (BRL) followed by phenol-chloroform extraction. Direct sequencing of the double-stranded PCR product was carried out using the Sequenase kit version 2.0 (United States Biochemicals).

## Results

*Cytogenetic studies.* Chromosomal abnormalities (deletions, translocations and isochromosomes) are summarized in Table I and representative examples illustrated in Figure 1. Frequent abnormalities (>80%) of chromosome 3p were present in SCLC, NSCLC and carcinoid lines, occasionally present in ExPuSC lines (40%) and absent from NSCLC-NE lines (0%). At chromosome 13q locus relatively frequent abnormalities were found in SCLC and carcinoid lines (68-75%), and less frequently in NSCLC (32%), NSCLC-NE and ExPuSC (20%) lines. At chromosome 17p abnormalities were relatively frequent in SCLC, NSCLC and carcinoids (>50%), and absent in the other tumor types.

*RFLP analysis of chromosome 3, 13, 17.* General information about the markers is presented in Table II, and a summary of the RFLP data is presented in Table III. Since matching normal tissue for each cell line were not available, loss of heterozygosity could not be determined directly. Instead, we pooled the data from each chromosomal location and compared the frequencies of heterozygosity in the unusual NE tumor cell lines with SCLC and NSCLC cell lines and with the general population (Table IV). The p values were computed from the asymptotic normal approximation to the binomial distribution. The normal frequencies of heterozygosity for each locus were obtained from the literature and were statistically similar to the incidences present in the normal tissues of SCLC and NSCLC patients (Table III). A representative selection of the blots are illustrated in Figure 2.

The frequencies of heterozygosity for the carcinoid lines closely resembled those of SCLC lines. The frequencies for NSCLC-NE lines were not significantly different from those of NSCLC lines, but were significantly different from those of SCLC lines. The RFLP patterns of the ExPuSC lines were variable, and did not closely resemble those of SCLC or NSCLC lines. of particular interest, the frequencies of heterozygosity at 3p loci in ExPuSC lines were similar to the general population, and significantly higher than the other tumor types.

*Oncogene abnormalities in unusual NE cell lines.* Data regarding analyses of p53 gene mutations, RB protein expression and K-ras point mutations are summarized in Table V.

Mutations and other abnormalities of the p53 gene were common in all lung cancer cell lines, but were universal in SCLC and carcinoid lines, and less common (74-83%) in

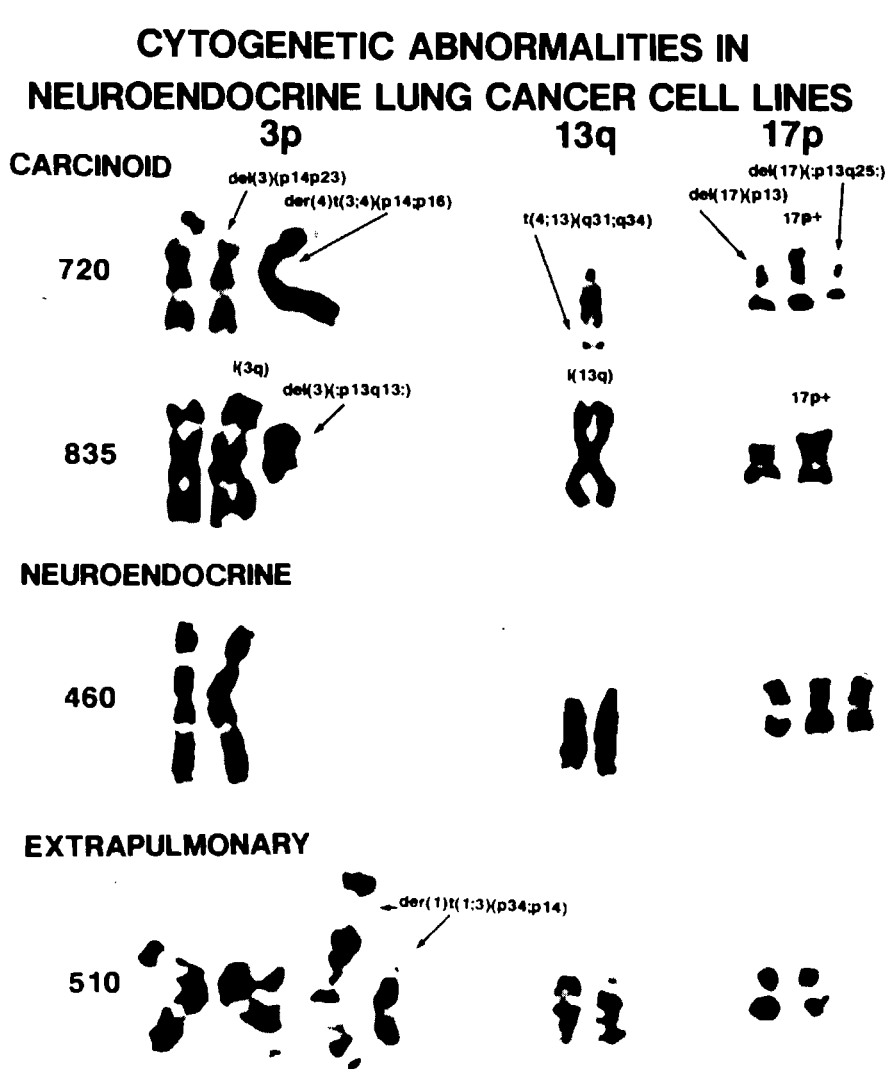


Figure 1. *Cytogenetic abnormalities in neuroendocrine lung cancer cell lines. Representative karyotypes of chromosomes 3, 13, 17 for cell lines are depicted. The cell lines are: NCI-H-720 and NCI-H835 (atypical carcinoid), NCI-H-460 (NSCLC-NE), and NCI-H-510 (ExPuSC). Chromosomal abnormalities are indicated.*

Table II. *General information about probes used for RFLP studies.*

| Gene/locus | Chromosomal location | Probe name | Restriction enzyme | Allele sizes | Frequencies of heterozygosity* |
|---|---|---|---|---|---|
| YNZ 86.1 | 3p21 | D3S30 | MspI | 2.0/1.9 | 0.5 |
| DNF15S2 | 3p21 | pH3H2 | HindIII | 2.3/2.0 | 0.48 |
| EFD14S5.1 | 3P21 | D3S32 | TaqI | 6.0/4.0 | 0.46 |
| D3S2 | 3p14-21 | p12-32 | MspI | 2.9/1.3 | 0.42 |
| c-RAF-1 | 3p25 | p627 | BglI | 4.0/3.3 | 0.5 |
| D13S1 | 13q12-14 | p7F12 | MspI | 4.3/3.4 | 0.64 |
| D13S2 | 13q22 | p9D11 | MspI | 15/10 | 0.44 |
| YNZ22 | 17p13.3 | D17S30 | BamHI | VNIR | 0.86 |
| THH59 | 17q23-25.3 | D17S4 | PvuII | VNTR | 0.71 |

* In the general population

Table III. *RFLP analyses of lung cancer cell lines.*

| Cell line type | YNZ86.1 3p21 | DNF15S2 3p21 | EFD145.1 3p21 | D3S2 3p14-21 | c-RAF-1 3p25 | D13S1 13q12-14 | D13S2 13q22 | YNZ22 17p13.3 | THH59 17q23-25.3 |
|---|---|---|---|---|---|---|---|---|---|
| General population | 0.52 | 0.48 | 0.46 | 0.42 | 0.5 | 0.64 | 0.44 | 0.86 | 0.71 |
| SCLC | 0.02 | 0 | 0.02 | 0.02 | 0 | 0 | 0.09 | 0 | Not known |
| NSCLC | 0.18 | 0.25 | 0.09 | 0/09 | 0.18 | 0.22 | 0.43 | 0.31 | Not known |
| Carcinoid (n = 5) | 0 | 0 5 | 0 | 0 5 | 0.4 | 0 | 0 | 0% | 0.4 |
| NSCLC-NE (n = 6) | 0.17 | 0.5 | 0.17 | 0.17 | 0.5 | 0 | 0.5 | 0.33 | 0.17 |
| ExPuSC n = 4) | 0.5 | 0.75 | 0.25 | 0.5 | 0.75 | 0 | 0 | 0.5 | 0 |

Figures for the general population, SCLC and NSCLC are based on greater than 40 observations for each group..

Table IV. *Comparison of frequencies of heterozygosity in neuroendocrine cell lines with general population and SCLC and NSCLC cell lines.*

| | General population | SCLC | NSCLC |
|---|---|---|---|
| Chromosome 3p | | | |
| Carcinoid | p 1 <0.0001 | NS | p2 = 0.03 |
| NSCLC-NE | p1 = 0.045 | p2 < 0.0001 | p2 = 0.02 |
| ExPuSc | NS | p2 < 0.0001 | p2 < 0.0001 |
| Chromosome 13q | | | |
| Carcinoid | p1 = 0.003 | NS | p2 = 0.024 |
| NSCLC-NE | p1 = 0.02 | p2 = 0.0004 | NS |
| ExPuSc | p1 = 0.001 | NS | p2 = 0.046 |
| Chromosome 17p | | | |
| Carcinoid | p1 <0.0001 | p2 = 1.0 | NS |
| NSCLC-NE | p1 = 0.00045 | p2 < 0.0001 | NS |
| ExPuSc | p1 = 0.097 | p2 <0.0001 | NS |

Table V. *Frequencies of oncogene abnormalities* in lung cancer cell lines.*

| Tumor type (n) | p53 gene* | rb gene* | K-ras* |
|---|---|---|---|
| SCLC | 33/33 (100%) | 66/75 (88%) | 0/37 (0%) |
| NSCLC | 57/77 (74%) | 11/74 (14%) | 18/56 (32%) |
| Carcinoid | 4/4 (100%) | 1/6 (17%) | 1/4 (25%) |
| NSCLC-NE | 5/6 (83%) | 1/6 (17%) | 4/6 (67%) |
| ExPuSC | 4/5 (80%) | 4/5 (80%) | 0/5 (0%) |

* Number abnormal/number tested (%). For p53 gene, abnormal patterns detected by SSCP analysis of exons 5-8. Most abnormal patterns were confirmed by sequencing. For rb gene, expression of RB protien by Western blotting. Abnormal patterns were identified as described in Methods. For K-*ras* gene, base substitutions in codons 12, 13, or 61, as determined by designed RFLP method, and confirmed by direct sequencing.

NSCLC, NSCLC-NE and ExPuSC lines.

Abnormal patterns of RB protein expression as determined by Western blotting were frequent in SCLC and ExPuSC lines (88% and 80% respectively), but occurred at much lower frequencies in NSCLC, NSCLC-NE and carcinoid lines (14-17%).

Point mutations of the K-ras gene were absent in SCLC and carcinoid lines. The frequencies in NSCLC and carcinoid lines were similar (32% and 25% respectively) (Table V). The frequency in NSCLC-NE lines was high (67%).

## Discussion

Carcinoid, NSCLC-NE and ExPuSC tumors express the same pattern of NE cell differentiation as SCLC. However, it is not known whether these tumors share the same genetic alterations, although retention of chromosome 3p in ExPuSC has previously been demonstrated by molecular and cytogenetic studies (23), and RB protein patterns for some of the cell lines have been recently published (35). The studies reported herein were performed in an effort to understand the inter-relationships and pathogenesis of these unusual NE tumors. We have reported the establishment of cell lines from the three types of NE tumors, and had characterized their NE cell features, in vitro chemosensitivy profiles and MDR1 gene expression patterns (2,36,22). Because corresponding non-malignant tissues were not available from these cases, we pooled the RFLP data from each chromosomal location and compared the frequencies of heterozygosity in the unusual NE tumor cell lines with SCLC and NSCLC cell lines and with the general population. We have summarized the data presented in this report, along with our previously published data on MDR1 gene RNA

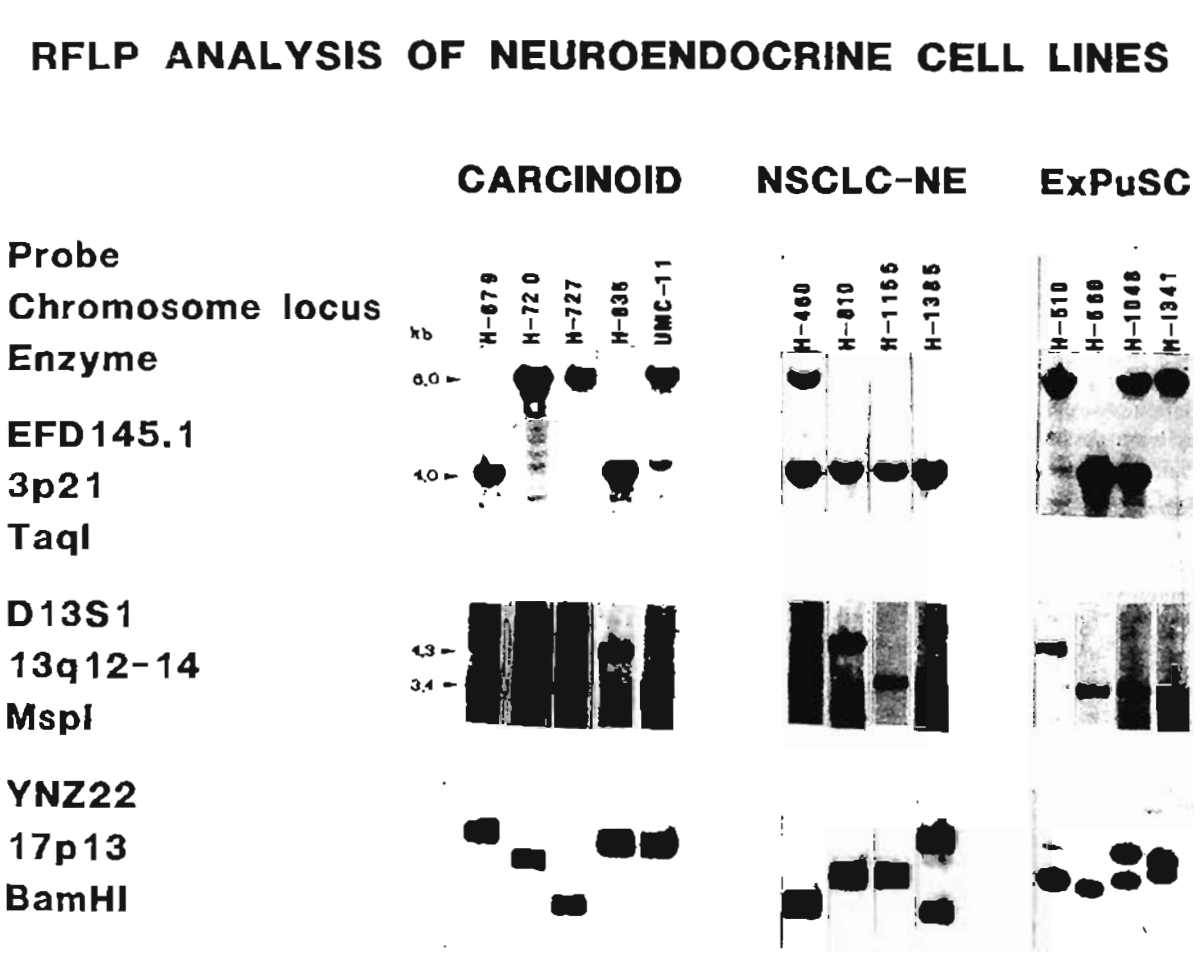


Figure 2. *Restriction fragment length polymorphism (RFLP) analysis of neuroendocrine lung cancer cell lines. Representative Southern blot hybridization patterns for 3 different polymorphic chromosomal loci are shown. EFD145.1 (3p21, TaqI), D13S1 (13q12-14, MspI), YNZ22 (17p13, BamHI) represent the probes, corresponding chromosomal locations and restriction enzymes used. Numbers to the left of autoradiographs indicated the molecular size of the two polymorphic alleles in kilobases. YNZ22 is a VNTR (variable number of tandem repeats) marker on chromosome 17p.*

expression and in vitro chemosensitivity (Table 6). It is obvious from these patterns, that these three NE tumor types represent distinct molecular entities, and differ from SCLC and NSCLC, as well as from each other.

Cytogenetic abnormalities and loss of genetic material at multiple loci on chromosome 3p are common in all forms of lung cancer (37). Presumably this region is the site of several (at least three) growth regulatory (tumor suppressor) genes. Peripheral lymphocytes of smokers demonstrate elevated expression of fragile sites at the cancer breakpoints including 3p14.2 (38). While these findings suggest that cigarette smoke may play a role in the loss of genetic material at this site, other mechanisms must exist, as 3p deletions are common in certain tumors not strongly associated with smoking, including breast and renal carcinomas (39,40). Chromosome 3p deletions are almost universal in SCLC and carcinoids, and frequent in NSCLC and NSCLC-NE. However, they occur only occasionally in ExPuSC. These differences may reflect the role of tobacco smoke and other airborne carcinogens in the pathogenesis of lung cancers. ExPuSC cancers arise at many sites throughout the body (other than the lungs), and exposure to tobacco smoke is unlikely to play a role in the pathogenesis of most of them.

Abnormalities of RB protein expression were common in SCLC and ExPuSC, and considerably less frequent in other forms of lung cancer. To some extent, these findings were reflected by the cytogenetic studies and the incidences of heterozygosity at chromosome 13q (the site of the rb gene). However, for carcinoids, there were considerable discrepancies in the results of these three assays. As previously mentioned, lack of DNA from corresponding normal tissues of the unusual NE cell lines made their interpretation less reliable.

Mutations of the p53 gene (located at 17p.13) were common in all types of lung cancers, although they were universal in SCLC and carcinoids. However, the incidences of cytogenetic abnormalities and were lower and more variable.

The incidences of K-*ras* mutations in the lung cancer lines showed interesting differences. To date, ras mutations have

Table VI. *Summary of properties of lung cancer cell lines.*

| Tumor type | Loss 3p | Loss 13q | Loss 17p | p53 mutations | Abnormal RB expression | ras gene mutations | MDR1 gene expression | Relative chemo-sensitivity |
|---|---|---|---|---|---|---|---|---|
| SCLC | Very common | Common | Common | Almost always | Very common | Never | Low | Very sensitive |
| NSCLC | Common | Occasional | Common | Common | Occasional | Occasional | Low | Resistant |
| Carcinoid | Very common | Very common | Occasional | Almost always | Occasional | Occasional | Relatively high | Very resistant |
| NSCLC-NE | Occasional | Occasional | Occasional | Very common | Occasional | Common | Relatively high | Very sensitive |
| ExPuSC | Occasional | Occasional | Occasional | Very common | Very common | Never | Low | Sensitive |

not been demonstrated in any SCLC tumor or cell line (41,42), and they were absent in ExPuSC lines. A subset of NSCLC tumors and lines (approximately 30%), especially adenocarcinomas and large cell carcinomas, have mutations (41,42). Carcinoid cell lines had a similar incidence (25%). However, two larger series, which analyzed fresh tumor samples, failed to find ras mutations in carcinoids and large cell neuroendocrine carcinomas (43,44). Of considerable interest, ras mutations were found in 4/6 (67%) of NSCLC-NE lines, all of which were derived from large cell or adenocarcinomas.

Our previously reported findings regarding MDR1 gene expression and chemosensitivity patterns of the various types of lung cancer cell lines are summarized in Table IV. Most SCLC and NSCLC lines (even those established from patients who had previously received chemotherapy) express low or undetectable levels of MDR1 (36). However, the use of a highly sensitive PCR-based quantitative assay demonstrated low expression levels in most cell lines (45). Thus both chemosensitive SCLC, and chemoresistant NSCLC expressed equally low levels. The ExPuSC (varying chemosensitivities) expressed low levels, while the highly chemoresistant carcinoids expressed. NSCLC-NE lines demonstrated the biggest discrepancy - they were highly chemosensitive, but expressed relatively high levels of MDR1. Thus, there was no correlation between chemosensitivy patterns and MDR1 expression. In addition, these patterns help confirm the unique phenotype of the five subtypes of lung cancer that we investigated.

In summary, despite the similarity of biological features in neuroendocrine lung cancer cell lines, our studies demonstrate that all three of the NE cell subtypes have their own distinctive genotypes and phenotypes, each having some similarities and dissimilarities with SCLC and NSCLC.

## Acknowledgements

We thank David Venzon for assistance with statistical analyses, David Carlone for performing two of the p53 gene analyses, and John Minna for encouragement and suprort.

## References

1 Gazdar AF, Carney DN, Russell EK, Sims HL, Baylin SB, Bunn PJ, Guccion JG and Minna JD: Establishment of continuous, clonable cultures of small-cell carcinoma of lung which have amine precursor uptake and decarboxylation cell properties. Cancer Res *40:* 3502-7, 1980.

2 Gazdar AF, Helman LJ, Israel MA, Russell EK, Linnoila RI, Mulshine JL, Schuller HM and Park JG: Expression of neuroendocrine cell markers L-dopa decarboxylase, chromogranin A, and dense core granules in human tumors of endocrine and nonendocrine origin. Cancer Res *48:* 4078-4082, 1988.

3 Gazdar AF, Tsai CM, Park JG, Ihde DC, Mulshine J, Carmichael J, Mitchell JB and Minna JD *In vitro* assays for predicting clinical response in human lung , cancer. *In:* J. D. Chapman, L. J. Peters and H. R. Withers (eds.), Prediction of Tumor Treatment Response, pp. 175-186. New York: Pergamon Press, 1989.

4 Linnoila RI, Mulshine JL, Steinberg SM, Funa K, Matthews MJ, Cotelingam JD and Gazdar AF: Neuroendocrine differentiation in endocrine and non-endocrine lung carcinomas. Amer J Clin Pathol *90:* 641-652, 1988.

5 Levenson RM, Ihde DC, Matthews MJ, Cohen MH, Gazdar AF, Bunn PA and Minna JD: Small cell carcinoma presenting as an extrapulmonary neoplasm: sites of origin and response to chemotherapy. J Natl Cancer Instl *67:* 607-612, 1981.

6 Remick SC, Hafez GR and Carbone PP: Extrapulmonary small cell carcinoma: A review of the literature with emphasis on therapy and outcome. Medicinel *66:* 457471, 1987.

7 Ibrahim NB, Briggs JC and Corbishley CM: Extrapulmonary oat cell carcinoma. Cancer *54:* 1645-61, 1984.

8 Carbone DP and Minna JD: The molecular genetics of lung cancer. Adv Int Med *37:* 153-171, 1992.

9 Whang-Peng J, Bunn PA, Kao SC, Lee EC, Carney DN, Gazdar AF and Minna JD: A non-random chromosomal abnormality, del 3p(14-23) in human small cell lung cancer. Cancer Genet Cytogenet *6:* 119-134, 1982.

10 Brauch H, Johnson B, Hovis J, Yano T, Gazdar A, Pettengill os, Graziano S, Sorenson GD, Poiesz BJ, Minna JD, Linehan M and Zbar B: Molecular analysis of the short arm of chromosome 3 in small-cell and non-small cell carcinoma of the lung. New Engl J Medicine *131:* 1109- 1113, 1987.

11 Kok K, Osinga J, Carritt B, Davis MB, van der Hout AH, van der Veen AY, Landsvater RM, de Leij LF, Berendsen HH, Postmus PE, Poppema S and Buys CH: Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. Nature *330:* 578-581, 1987.

12 Yokota J, Wada M, Shimosato Y, Terada M and Sugimura T: Loss of heterozygosity on chromosomes 3, 13, and 17 in small-cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. Proc Natl Acad Sci USA *84:* 9252-9256, 1987.

13 Johnson BE, Sakaguchi AY, Gazdar AF, Minna JD, Burch D, Marshall A and Naylor SL: Restriction fragment length polymorphism studies show consistent loss of chromosome 3p alleles in small cell lung cancer patients' tumors. J Clin Invest *82:* 502-507, 1988.

14 Naylor SL, Marshall A, Johnson BE, Minna JD, Gazdar AF, Whang-Peng J, Lee EC and Sakaguchi AY: Chromosome 3p in small cell lung cancer. Lung Cancer *4:* 117-120, 1988.

15 Mori N, Yokota J, oshimura M, Cavenee WK, Mizoguchi H, Noguchi M, Shimosato Y, Sugimura T and Terada M: Concordant deletions of chromosome 3p and loss of heterozygosity for chromosomes 13 and 17 in small cell lung carcinoma. Cancer Res *49:* 5130-5135, 1989.

16 Graziano SL, Pfeifer AM, Testa JR, Mark GE, Johnson BE, Hallinan EJ, Pettengill OS, Sorenson GD, Tatum AH, Brauch H and *et al:* Involvement of the RAF1 locus, at band 3p25, in the 3p deletion of small-cell lung cancer. Genes Chromosomes Cancer *3:* 283-93, 1991.

17 Lukeis R, Irving L, Garson M and Hasthorpe S: Cytogenetics of non-small cell lung cancer: analysis of consistent non-random abnormalities. Genes Chromosomes Cancer *2:* 116-124, 1990.

18 Miura I, Siegfried JM, Resau J, Keller SM, Zhou JY and Testa JR: Chromosome alterations in 21 non-small cell lung carcinomas. Genes Chromosomes *2:* 328-338, 1990.

19 Whang-Peng J, Knutsen T, Gazdar A, Steinberg SM, Oie H, Linnoila I, Mulshine J, Nau M and Minna JD: Nonrandom structural and numerical chromosome changes in non-small-cell lung cancer. Genes, Chromosomes Cancer *3:* 168-188, 1991.

20 D'Amico D, Carbone DP, Johnson BE, Meltzer SJ and Minna JD: Polymorphic sites within the MCC and APC loci reveal very frequent loss of heterozygosity in human small cell lung cancer. Cancer Res *52:* 1996-1999, 1992.

21 Olopade OI, Jenkins RB, Ransom DT, Malik K, Pomykala H, Nobori T, Cowan JM, Rowley JD and Diaz MO: Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res *52:* 2523-9, 1992.

22 Gazdar AF, Kadoyama C, Venzon D, Park J-G, Tsai C-M, Linnoila RI, Mulshine JL, Ihde DC and Giaccone G: The association between histological type and neuroendocrine differentiation on drug sensitivity of lung cancer cell lines. J. Natl. Cancer Inst. Monogr *13:* 23-29, 1992.

23 Johnson BE, Whang-Peng J, Naylor SL, Zbar B, Brauch H, Lee E, Simmons A, Russell E, Nam MH and Gazdar AF: Retention of chromosome 3 in extrapulmonary small cell cancer shown by molecular and cytogenetic studies. J Natl Cancer Inst *81:* 1223-1228, 1989.

24 Carney DN, Gazdar AF, Bepler G, Guccion J, Marangos PJ, Moody TW, Zwcig MH and Minna JD: Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res *45:* 2913-2923, 1985.

25 Harnden DG and Klinger HP. An International System for Cytogenetic Nomenclature. New York: March of Dimes Birth Defects Foundation. 1985.

26 Carrit B, Welch HM and Parry-Jones NJ: Sequences homologous to the human D1S1 locus present on human chromosome 3. Am J Hum Genet *38:* 428-436, 1986.

27 Leppert M, O'Connel P, Nakamura Y, cartwright P, Lathrop M, Lalouel JM and White R: Two linkage groups on chromosome 3. Cytogenet Cell Genet *46:* 648, 1987.

28 Seizinger BR, Rouleau GA, Ozelius LJ, Lane AH, Farmer GE, Lamiell JM, Haines J, Yuen JW, Collins D and Majoor-Krakauer D: Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. Nature *332:* 268-9, 1988.

29 Barker D, Schafer M and White R: Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell *36:* 131-8, 1984.

30 Cavanee W, Leach R, Mohandas T, Pearson P and White R: Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. Aml J7 Human Genetlt *36:* 10-24, 1984.

31 Nakamura Y, Culver M, Gillilan S, O'Connel P, Leppert M, Lathrop GM, Lalouel JM and White R: Isolation and mapping of a polymorphic DNA sequence pYNZ86.1 on chromosome 3 (D3S30). Nucleic Acids Res *15:* 10079, 1987.

32 Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E and White R: Variable number of tandem repeat (VNTR) markers for human gene mapping. Science *235:* 1616-1622, 1987.

33 Towbin H, Staehlin T and Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Oric Nat Acad Sci USA *76:* 4350-4354, 1979.

34 Mitsudomi T, Steinberg SM, Nau MM, Carbone D, D'Amico D, Bodner S, Oie HK, Linnoila RI, Mulshine JL, Minna JD and Gazdar AF: p53 gene mutations in non-small-cell lung cancer cell lines and their correlation with the presence of ras mutations and clinical features. Oncogene *7:* 171-80, 1992.

35 Shimizu E, Coxon A, Otterson GA, Steinberg SM, Kratzke RA, Kim YH, Fedorko J, Oie H, Johnson BE, Mulshine JL, Minna JD and Kaye FJ: RB protein status and clinical correlation from 172 cell lines representing lung cancer, extrapulmonary small cell carcinoma and mesothelioma. Oncogene, in press 1994.

36 Lai SL, Goldstein LJ, Gottesman MM, Pastan I, Tsai CM, Johnson BE, Mulshine JL, Ihde DC, Kayser K and Gazdar AF: MDR1 gene expression in lung cancer. J Natl Cancer Instl *81:* 1144-1150, 1989.

37 Brauch H, Tory K, Kotler F, Gazdar AF, Pettengill OS, Johnson B, Graziano S, Winton T, Buys CH, Sorenson GD, Minna J and Zbar B: Molecular mapping of deletion sites in the short arm of chromosome 3 in human lung cancer. Genes Chromosomes Cancer *1:* 240-246, 1990.

38 Kao SC, Fine RL, Whang-Peng J, Lee EC and Chabner BA: Increased fragile sites and sister chromatid exchanges in bone marrow and peripheral blood of young cigarette smokers. Cancer Resl *47:* 6278-6282, 1987.

39 Zbar B ; Chromosomal deletions in lung cancer and renal cancer. *In:* V. DeVita, S. Hellman and S. A. Rosenberg (eds), Important Adv. Oncol., pp. 41-60. Philadelphia: J. B. Lippincptt, 1989.

40 Callahan R, Gallahan D, Smith G, Cropp C, Merlo G, Diella F, Liscia D and Lidereau R: Frequent mutations in breast cancer. Ann N Y Acad Sci USA *698:* 21-30, 1993.

41 Rodenhuis S and Slebos RJ: The *ras* oncogenes in human lung cancer. Am Rev Respir Disl *142:* 527-530, 1990.

42 Mitsudomi T, Viallet J, Mulshine JL, Linnoila RI, Minna JD and Gazdar AF: Mutations of *ras* genes distinguish a subset of non-small-cell lung cancer cell lines from small-cell lung cancer cell lines. Oncogene *6:* 1353-1362, 1991.

43 Rodenhuis S and Slebos RJ: Clinical significance of ras oncogene activation in human lung cancer. Cancer Res *52:* 2665s-2669s, 1992.

44 Wagner SN, Muller R, Boehm J, Putz B, Wunsch PH and Hofler H: Neuroendocrine neoplasms of the lung are not associated with point mutations at codon 12 of the Ki-*ras* gene. Virchows Arch B Cell Pathol *63:* 325-9, 1993.

45 Noonan KE, Beck C, Holzmayer TA, Chin JE, Wunder JS, Andrulis IL, Gazdar AF, Willman CL, Griffith B, Von Hoff DD and Roninson IB: Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci USA *87:* 7160-7164, 1990.

*Received December 8, 1994*
*Accepted January 11, 1995*